Biochemical Oxidation of Dairy Wastes. VII. Purification, Oxidation, Synthesis and Storage

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Oxidation and assimilation of waste by aerated sludge were emphasized in our studies summarized in a microbiological process report (20). Aeration of a synthetic waste skim milk with sufficient agitation led to two premises. First, there occurs a rapid assimilation and conversion of the available C.O.D. into microbial cells or sludge. During this period, 37.5% of the C.O.D. is completely oxidized to CO_2 while cell material is synthesized (10). Second, the cell material is subsequently oxidized during catabolism by endogenous respiration. Sludge oxidation continues at the rate of 1% per hour (8) and higher according to pilot plant studies at Pennsylvania State University (11).

Thus, with vigorous agitation and aeration, 1000 p.p.m. skim milk solids were changed in 6 hours to additional 500 p.p.m. sludge solids by the 500 p.p.m. sludge present as seed (20). Increasing sludge concentration reduces the time required for the first step. Such assimilation and oxidation were stoichiometrically represented by the following hypothetical equation of synthesis:

$$8(CH_2O) + C_8H_{12}N_2O_3 + 6\ O_2 \rightarrow 2C_5H_7NO_2 + 6\ CO_2 + 7\ H_2O.$$

This equation shows that 8 moles of lactose carbon and 8 moles of casein carbon required 6 moles of oxygen for conversion to microbial cells with the formation of 6 moles of carbon dioxide. Respirometer studies showed that lactose and casein are oxidized at the same rate based on their oxygen demands (7).

In our studies it was more feasible to calculate the starting C.O.D. from previously determined values for the sludge and synthetic wastes. Analytical values for the mixtures gave apparent discrepancies that were attributed to sampling techniques or to rapidity of oxidation. Later, Kountz observed an almost instantaneous decrease in soluble C.O.D. when dairy waste was mixed with sludge in his pilot plant investigations (13). Eckenfelder noted a similar occurrence and applied this

observation in the high rate activated sludge treatment process he developed for the disposal of fruit wastes (3).

Purification and oxidation of some industrial wastes reported by Gellman and Heukelekian showed great differences between purification and oxidation (5). The importance of several factors was determined such as the amount of sludge, quantity and type of waste, temperature, acclimatization and sludge age. The average rate of purification was 3.9 times greater than the rate of oxidation, while that for milk waste was 6.5 times greater.

That study led to the report of Hoover, Jasewicz and Porges presented at the 9th Industrial Waste Conference (9) in which the average rate of purification was 10 times that of oxidation when 1000 p.p.m. skim milk and 1000 p.p.m. sludge were vigorously aerated in a fermentor. Calculations based on the equation of synthesis showed that the rate of purification should be 2.67 times that of the rate of oxidation if all available oxidizable materials were used. This factor was derived from the assimilation equation in which 6 of the available 16 carbons were completely oxidized, while 10 were used for cell synthesis. Thus the removal of organic matter as C.O.D. during cell synthesis or assimilation would be 16/6 or 2.67 times that oxidized. Purification or removal of C.O.D. greater than this value indicated nonoxidative accumulation or storage or adsorption of the excess amount.

Investigations on this accumulated stored material were continued in order to determine its effect on oxygen demand and to obtain information on the storage products. Some of the results were reported in part at the conference on biological waste treatment at Manhattan College (21). Additional results are reported in this presentation.

PROCEDURE

Previous investigations followed purification in one vessel and oxidation in a separate respirometer. A simple closed aerator (19) permitted both changes to be followed on one mixture. Oxygen uptake was followed by measuring the CO_2 evolved and fixed by barium hydroxide. Purification was determined by measuring the C.O.D. of the mixture by means of a rapid chromate method (22). Sludge solids were estimated by multiplying their C.O.D. by 0.8, since a unit of sludge averaged 1.25 units C.O.D. (7).

Each aerator contained in a final volume of 650 ml, 1000 p.p.m. sludge solids, 1125 p.p.m. skim milk C.O.D. and 47 p.p.m. soluble C.O.D. present with the sludge. The aerator was held at the indicated

temperature and CO_2-free air was passed through at the rate of 325 ml per vessel per minute. Mechanical agitation was omitted.

The seed sludge used for these tests was an aerated sludge developed for many days in the fermentor at 30° C. Earlier observations showed that a non-acclimated sludge would give greater differences between purification and oxidation when placed at a lower temperature.

The aerators were held at 2°, 10°, 20° and 30° C. Since air-agitation was not supplemented by mechanical agitation, activity was slower than that in the vigorously agitated aerator that required only 3

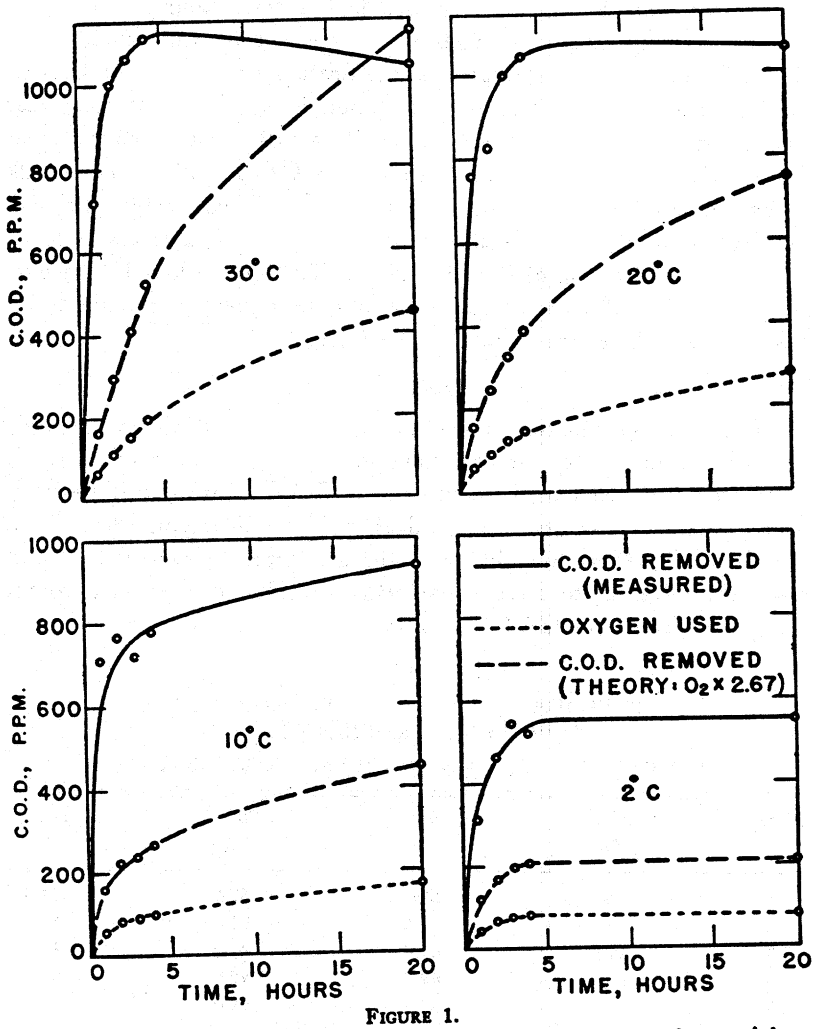

FIGURE 1.

Results of the investigations on the oxidation of accumulated material.

hours to enter the purely endogenous phase. Figure 1 gives the plotted results of these experiments.

C.O.D. Removal or Purification

Removal of C.O.D. varied with the temperature except during the first hour. Probably, the temperatures had not yet taken effect, since the aerating mixtures required 30 to 40 minutes to reach the surrounding temperature. The greatest C.O.D. removal or purification was obtained in 4 hours at 30° C. Considerable purification occurred even at 2° C. with 48% removal of the soluble milk C.O.D. At this low temperature activity practically ceased by the third hour.

At all temperatures, changes were apparent by the second hour. Table I summarizes the results on purification or removal of C.O.D. from solution. The degree of purification in 4 hours was somewhat greater at 30° C. than at 20° C., while at 10° it dropped to about 70%. The calculations were based on the C.O.D. of the skim milk to be removed. Based on the weight of the original sludge, the high purification ability of active sludge is apparent. In 1, 2, 3 and 4 hours at 30° C., 1000 p.p.m. of sludge removed 720, 1000, 1065, 1105 p.p.m. of C.O.D. At 20° C. purification was almost as great, but at the lower temperatures there was a marked decrease.

Oxidation

The synthesis equation shows that the oxygen utilized or the CO_2 evolved is a measure of the oxidizable material used for energy and cell structure. The calculated quantities of C.O.D. during cell synthesis or assimilation thus used were calculated by multiplying the oxygen used by 2.67. These are plotted as the dash lines in Figure 1. In all cases, the distances to these curves were considerably less than to the actual purification or C.O.D. removal curves.

TABLE I

PURIFICATION OR C.O.D. REMOVAL AT DIFFERENT TEMPERATURES

(1000 P.P.M. SLUDGE AND 1125 P.P.M. SKIM MILK C.O.D.)

Time Hour	2° C. %	10° C. %	20° C. %	30° C. %
1	27.6	63.1	67.9	64.0
2	41.3	67.6	73.4	89.2
3	48.3	64.6	89.0	94.6
4	46.4	69.2	92.9	98.3
20	49.1	83.5	95.0	92.4

Table II contains derived data showing the portions of the available C.O.D. calculated as used for energy and cell structure during assimilation. In the first 4 hours relatively small amounts of the available C.O.D. were used in the process of cell synthesis. As aeration continued, cell formation increased and a greater portion of the material removed from solution was used for this purpose. In 20 hours at 30° C. some of the original cell material was apparently being oxidized.

Temperature effects were noticeable. At 30° C., the theoretical amount of C.O.D. used, as obtained by extrapolation, would equal the C.O.D. removed by purification in about 10 hours under the conditions of these experiments. Extrapolation of the 20° C. data showed about 35 hours would be required. At 10° C., about 70 hours would be needed, while at 2° C. action had practically ceased. Considerable unoxidized material is still available for cell synthesis during the early period of purification.

Cell Formation or Synthesis

Aerated sludge is a mixture of heterogenous microorganisms. In the presence of food indicated by C.O.D. additional cells are formed by the process of reproduction. The amount of such cell material actually converted from the C.O.D. may be calculated from the oxygen required by means of the equation of synthesis. The equation shows that 10/6 or 1.67 times the oxygen utilized gives the C.O.D. converted to cell material. These values may be obtained also by taking 62.5% of the values shown in Table II.

TABLE II

C.O.D. USED FOR CELL AND ENERGY CALCULATED FROM O_2 USED x 2.67

(1000 P.P.M. SLUDGE AND 1125 P.P.M. SKIM MILK C.O.D.)

Time Hour	2° C. %	10° C. %	20° C. %	30° C. %
1	10.3	14.3	14.2	14.4
2	14.9	19.9	22.2	26.0
3	17.4	21.3	29.4	36.5
4	18.0	24.0	34.9	46.5
20	18.8	40.2	66.4	107.2

The actual amount of C.O.D. found as true cell material is only a small fraction of that available (Table III), and increases with time

and temperature. Examination of the data in Tables I and III show that at 2° C. a little over one-fifth of the C.O.D. removed from the solution in 4 hours may be considered fixed cell material. This was true at 10° C. and 20° C., also. At 30° C., about 30% of purification is actual cell formation at this time. By 20 hours, the values had changed to 71% at 30° C. and 33% at 20° C. Apparently endogenous respiration was the major activity at the higher temperature at the 20 hour testing. At lower temperatures, longer periods are apparently needed to observe the effect of endogenous respiration.

TABLE III

C.O.D. USED FOR CELL FORMATION, CALCULATED FROM O_2 USED x 1.67

(1000 P.P.M. SLUDGE AND 1125 P.P.M. SKIM MILK C.O.D.)

Time Hour	2° C. %	10° C. %	20° C. %	30° C. %
1	6.4	8.8	8.9	9.0
2	9.2	12.4	13.9	16.2
3	10.8	12.8	18.4	22.8
4	11.2	15.0	21.8	29.1
20	11.7	25.1	41.5	67.1

C.O.D. Oxidized

The C.O.D. oxidized to CO_2 was low. The percentages of the available C.O.D. completely oxidized are tabulated (Table IV). In only one case does the C.O.D. oxidized exceed the theoretical value of 37.5%. At 30° C., in 20 hours, the value is greater, showing oxidation of the sludge itself.

TABLE IV

C.O.D. OXIDIZED TO CO_2

(1000 P.P.M. SLUDGE AND 1125 P.P.M. SKIM MILK C.O.D.)

Time Hour	2° C. %	10° C. %	20° C. %	30° C. %
1	3.9	5.4	5.3	5.4
2	5.7	7.5	8.3	9.8
3	6.6	8.5	11.0	13.7
4	6.8	9.0	13.1	17.5
20	7.1	15.1	24.9	40.1

C.O.D. Stored

According to our premises, the C.O.D. removed during purification serves various purposes. A part is completely oxidized while a part produces cell substance during true assimilation. The remaining portion is unassimilated but is stored or adsorbed. Significant amounts of the C.O.D. exceeding that used for cell formation were stored (Table V). The figures in this table are derived data, obtained by subtracting those in Table II from Table I.

TABLE V

C.O.D. STORED BY AERATING SLUDGE

(1000 P.P.M. SLUDGE AND 1125 P.P.M. SKIM MILK C.O.D.)

Time Hour	2° C. %	10° C. %	20° C. %	30° C. %
1	17.3	48.8	53.7	49.6
2	26.4	47.7	51.2	63.2
3	30.9	43.3	59.6	58.1
4	27.4	45.2	58.0	51.8
20	30.3	43.3	28.6	−15.8

During the early assimilative phase of growth a great deal of available C.O.D. was stored. The storage ability varied with the temperature. Based upon the original 1000 p.p.m. sludge present, there was an apparent storage of 716 p.p.m. C.O.D. by the sludge at 30° C. in 2 hours. However, if we add the new cell material from Table III this storage ability is 620 p.p.m. C.O.D. per 1000 p.p.m. cells. At 20° C. in 3 hours, the value is slightly less, being 555 p.p.m. per 1000 p.p.m cells. Lesser storage ability was found at lower temperatures.

This stored material has an oxygen demand that must be satisfied to maintain aerobic conditions. Part will be converted to cell, part will be oxidized. It is interesting to note that when this stored material was calculated on a C.O.D. basis or a solids basis, a well aerated endogenous sludge was able to store 50% of its own weight. Therefore a sample of material removed after 2 hours at 30° C. would contain about 33% of its weight as readily oxidizable material.

Form of Storage

It is generally considered that sludge removes C.O.D. by a simple surface adsorption. Our work with skim milk showed that little or none of the soluble C.O.D. adhered to the surface. Lactose was almost

completely absent. Indications were that the carbohydrate was converted to storage product upon entering the cell.

Larger quantities of cells were needed to obtain more information about this unassimilated or stored material. An active sludge was prepared at 30° C. in the large aerator and then placed in a 10° C. water bath. (At the time this was done, it was believed that this lower temperature would favor higher storage because of decreased metabolic activity.) Skim milk was added; agitation and aeration were continued. At 5 hours and 24 hours, 6 liters of the mixture were chilled with ice and passed through a centrifuge. The cells were dispersed in chilled physiological saline solution (0.9% NaCl) and centrifuged again. The harvested cells were lyophilized and used for study.

Paper Chromatography

Half gram samples were weighed into thick walled 58 ml. centrifuge tubes and treated successively in a boiling water bath for 30 minutes with 25 ml. water, 25 ml. 50% alcohol and 2 hours with 2% HCl. Glass tube air condensers were used for closures. After each treatment, the extracts were recovered by centrifugation. The proximate contents of various extracts were described in a study on mycelium (18). The alcohol extract was deionized and it and the water extract were evaporated to dryness under vacuum. The dry materials were dissolved in one ml. water. Papergram analyses were performed for sugar according to the methods of Porter, Hoban and Willits (23) and for hexoseamines according to Partridge (17), as outlined in "Paper Chromatography", by Block, LeStrange and Zweig (1).

These chromatographic studies of sludge showed that at 5 hours, the water extract contained only a trace of glucose, the alcohol extract had more of the glucose, maltose and four hexoseamines, while the HCl extract was rich in glucose and had some pentose. The 24-hour sludge gave extracts having different papergrams. Sugars were absent in the water extract. Only traces of glucose and one hexoseamine were found in the alcohol extract, while the HCl extract was rich in glucose only.

The easily soluble substances present in the sludge during rapid assimilation finally disappear leaving a more stable substance requiring acid hydrolysis to yield glucose. Lactose and its hydrolytic product, galactose, were completely absent. The soluble sugars were changed evidently into other chemical oxygen demanding substances.

Glycogen Content

Microorganisms are known to contain glycogen as do muscle, liver and other animal tissues. Edible fungi had 12.6% (24), filamentous

fungi associated with root rot contained as much as 36.7% (4), yeast 1 to 8% (2) and even greater amounts. Ciliates had 23% (15) while the amount of glycogen reported in bacteria varied from 8% to 50% (14).

Glycogen determinations (12) of the sludge harvested at 5 hours showed 19.3% which decreased to 8.8% in 24 hours. Another sludge grown at 30° C. had 15.3% glycogen-like substances at 1 hour and only 5.5% after 36 hours aeration and agitation.

Glycogen was the major storage form. This may be inferred by calculating the composition of the sludge grown at 10° as shown in the tables. The C.O.D. of 1000 p.p.m. sludge averages 1250 p.p.m. (7); 15% of 1125 p.p.m. skim milk C.O.D. was converted to new cells (Table III) to give 169 pp.m. C.O.D. or a total of 1419 p.p.m. cell C.O.D. The stored C.O.D. was 45.2% (Table V) of the 1125 p.p.m. C.O.D. available or 509 p.p.m. C.O.D. The total C.O.D. of the original sludge, new cells and stored material totaled 1928 p.p.m. of which 509 p.p.m. or 26.4% was storage C.O.D. This calculated value is not too divergent from the 19.3% glycogen found under conditions of vigorous aeration in the large fermentor. These results were corroborated by a recent publication in which the content of polyglucose of glycogen nature was determined in *Escherichia coli* during the first hours of growth (16). In shake-flask experiments using sodium lactate as the only carbon source, the glycogen reached almost 13% of the dry weight of the cells in 30 minutes. The original glycogen content was 1% and in 6 hours it reached this low value again showing that the synthesized glycogen was used by the growing organisms.

DISCUSSION

Purification of a waste involves at least three distinct processes: oxidation, synthesis and storage. At 30° C., in 2 hours, 1000 p.p.m. sludge removed 89% of 1125 p.p.m. available C.O.D. or 1000 p.p.m. Only 11% of the C.O.D. removed was oxidized to CO_2, 18% was converted to cell substance and 71% was stored. Practically the same proportions were found at 20° in 3 hours. The stored material continued to have a high oxygen demand that must be satisfied since each unit of C.O.D. requires a unit of oxygen. When newly formed sludge was also taken into consideration, sludge storage is somewhat less as 1000 p.p.m. of cells stored 620 p.p.m. C.O.D. In other words, about one-third of the sludge C.O.D. was in the form of an available carbohydrate. As aeration continued this storage carbohydrate decreased.

Glycogen was the major storage product of this sludge. The simple C.O.D. products removed from solution were converted to the insoluble but readily available glycogen. This glycogen was utilized by the sludge and tended to disappear as the sludge completed its true assimilation phase of growth and entered the endogenous phase.

This purification and storage ability may have practical application. Rapid and complete purification may be anticipated if 3,000 p.p.m. of well aerated sludge in the endogenous state are mixed with 1000 p.p.m. of soluble C.O.D. The cells now loaded with glycogen and other products may be removed leaving a clear effluent. However, aeration of the sludge must continue in order to assure rapid oxidation of the stored C.O.D. The depleted or starved cells may then be reused for further purification of wastes.

The amount of nitrogen that must be added to a nitrogen deficient waste may also be determined. The hypothetical cells, $C_5H_7NO_2$, contain 12.4% nitrogen. When 1000 p.p.m. of these cells store 620 p.p.m. of glycogen C.O.D. the nitrogen content drops to 7.5%. Hence, complete removal of C.O.D. may be anticipated if there is sufficient nitrogen present to yield a sludge containing about 7% of this element. Helmers, Frame, Greenberg and Sawyer (6) showed a B.O.D. to nitrogen ratio of 19 to 1 was desirable. This calculates as a C.O.D. to N ratio of 28 to 1. If we consider that 62.5% of the available C.O.D. is used in cell assimilation and that this C.O.D. probably represents carbohydrates, the calculated nitrogen content of the sludge will be about 6.9%, a value approximating that estimated for the hypothetical cells when they are loaded with stored material.

Preliminary experiments suggest using this ability of microorganisms to store and to continue activity within a wide nitrogen range for the removal of low nitrogen-containing wastes in a fill and draw or continuous method without nitrogen supplementation. Studies are continuing on this phase of our problem.

SUMMARY

Rapid purification or removal of C.O.D. from a waste by an active sludge consists, at least, of three distinct processes: oxidation, synthesis and storage. The assimilation equation permitted the amounts used for assimilation and oxidation to be determined from oxygen utilization. Non-assimilated C.O.D. or storage was determined by subtracting the above values from the total C.O.D. removed by purification. C.O.D. used during assimilation for cell synthesis and energy was determined

by multiplying the oxygen utilized by 2.67. The factor 1.67 was used to obtain the C. O. D. converted to cell material.

Apparently 1000 p.p.m. sludge stored 716 p.p.m. skim milk C.O.D. at 30° C. in 2 hours with some formation of new cells. The sludge cells stored half their own weight as oxidizable material, if the new cells are considered. Glycogen was the main storage product since as much as 19% was found in the sludge after 5 hours. Storage glycogen was rapidly oxidized as very little was present in endogenous cells.

Data on storage and cell synthesis of sludge are presented for 2°, 10°, 20° and 30° C. in graphs and tables.

The ability of microorganisms to store and oxidize wastes within a wide nitrogen range may find application in rapid purification and subsequent oxidation of low nitrogen-containing wastes.

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